# *Johnsprentia copemani* gen. nov., sp. nov. (Haemoproteidae), a parasite of the flying-fox, *Pteropus alecto* (Pteropidae) from Queensland

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## ABSTRACT

Black flying foxes (*Pteropus alecto* Temminck, 1837) captured in Townsville, Queensland, are parasitised by a new species of Haemoproteidae which differs morphologically and in its tissue localisation, having highly distinctive gametocytes and tissue forms in the lungs, which are described here. It cannot be accommodated in any of the genera of the Haemoproteidae known from mammals and is here named *Johnsprentia copemani* gen. nov., sp. nov. ❐ *Haemoproteidae, Pteropus alecto, Johnsprentia copemani, new genus, new species, Queensland.*

Knowledge of the haemoproteid parasites of native Australian mammals is limited, with all those known to date being reported by O'Donoghue & Adlard (2000). Studies on flying foxes are particularly limited although one species, *Hepatocystis pteropi* (Breinl 1913), has been reported from a number of species of flying foxes (Mackerras 1959).

We had the opportunity to examine the haemoproteid parasites of several black flying foxes, *Pteropus alecto* Temminck, 1837, over a period of time in captivity. It was apparent that, depending upon the individual, one to three species of haemoproteids were present, at differing levels, in their blood. Histological examination of internal organs using serial sections also revealed three types of schizonts which differed in their size, their morphology and their localisation.

We describe here the gametocytes and schizonts of one of these haemoproteid species. It cannot be accommodated in any of the known genera. Here we describe and name it as *Johnsprentia* gen. nov. *copemani* sp. nov. after two noted Australian parasitologists, J.F.A. Sprent and D.B. Copeman. The remaining two species will be the subject of a later paper.

## MATERIALS AND METHODS

Eleven *Pteropus alecto* Temminck (1837) captured in Townsville using a mist net and exhibiting a parasitaemia with Haemoproteidae, were tran-

sported to the Muséum National d'Histoire Naturelle, Paris, shortly after their capture, arriving on the 15 December 1978 and the 07 June 1979 respectively. Blood samples from each animal were collected by pricking the radial vein and smeared onto a slide, air dried quickly, fixed with absolute methanol and stained by Giemsa stain (8% in buffer phosphate, pH7.4). They were examined over a period of several months. At autopsy, internal organs were fixed in Carnoy's fluid and serial sections of each organ were stained by the giemsa-colophonium method (Bray & Garnham 1962; Garnham 1966) and examined for tissue stages of the parasites. Type material has been deposited in the Queensland Museum, Brisbane (QM) and the Muséum National d'Histoire Naturelle (MNHN), Paris.

## SYSTEMATICS

Phylum: Apicomplexa (Sporozoa)

Class: Coccidea

Order: Haemosporida

Family: Haemoproteidae

### *Johnsprentia* gen. nov.

**Definition.** Haemoproteidae with elongated schizonts, in the lungs of Megachiroptera, not producing colloid, gametocytes in blood films with a nucleus apparently adherent to the margin of the parasite and with peripheral regions denser than the centre.

**Type Species.** *Johnsprentia copemani* sp. nov.

**Etymology.** Named after the late Professor John F.A. Sprent, formerly Professor of Parasitology at the University of Queensland.

### **Johnsprentia copemani** sp. nov. (Figs 1-2)

**Material.** HOLOTYPE. Histological section of schizont in lung of *Pteropus alecto* no. 409XF autopsied on 29/06/1979 in Paris (origin: Townsville, Queensland, Australia, slide deposited in QM No. G465432, illustrated in Fig. 2B). PARATYPES: a) One microgametocyte marked on a blood smear from the same bat, collected on the same date, deposited in QM No. G465433; b) a blood smear and histological sections of schizonts from the lung of *Pteropus alecto* no. 408XF and 20HD, deposited in MNHN PXX 201-208.

**Etymology.** Named after our colleague the late Dr. D. Bruce Copeman who helped capture flying foxes in his garden in Townsville.

**Description.** *Gametocytes.* Young forms or those which have just reached maturity are described from an animal (408XF) parasitised only by *Johnsprentia* (Fig. 1A-1H). This animal, on its arrival in Paris, had only ring-forms and trophozoites. It was examined daily until autopsy 21 days later. The immature parasites developed slowly and on the day of autopsy, several fully-developed gametocytes were observed. More mature gametocytes were found in other animals in which additional species of parasite were present (Fig. 1I-M). The young trophozoites are round or oval with a thin, arcuate nucleus along one side, sometimes with two unequal masses of chromatin. In young trophozoites, there are frequently fine cytoplasmic projections (Fig. 1B-D), which are absent in older forms. The cytoplasm is clear, pale-blue and with numerous poorly-defined vacuoles, some vacuoles being larger and better defined with fine grains of pigment. When the gametocyte reaches two-thirds the volume of the erythrocyte, it is spherical, clear in the centre, denser around the periphery, with the cytoplasm and most of the pigment displaced towards the periphery. The centre has scattered inconspicuous vacuoles and fine,


FIG. 1. Drawings of the gametocytes of *Johnsprentia copemani* sp. nov. stained with Giemsa. A, E, F, young gametocytes; B-D, young gametocytes with fine cytoplasmic prolongations; G-I, K, microgametocytes; J, old microgametocyte; L-M, macrogametocytes; N, O, normal red blood cell.

scattered grains of pigment. At this stage, it is difficult to differentiate microgametocytes and macrogametocytes (Fig. 1E-G). The fully developed microgametocytes are highly chromophilic, purple, with a dark, dense periphery (Fig. 1H, I, K). This is not due to overstaining as young forms in the same film do not stain in the same manner. As with the younger forms, small, scarcely visible vacuoles are scattered through the cytoplasm and the fine pigmentation is found mainly around the periphery. Old microgametocytes are often surrounded by a reddish ring. The nucleus is always peripheral, forming an elongated arc around the border. The macrogametocytes (Fig. 1M, N) are generally clearer than the microgametocytes, with a smaller nucleus. The red globular surrounding layer is of normal size, rarely slightly larger, of usual colour and is always visible around the parasite.

*Schizonts.* The schizonts are described from bat number 408 XF (Fig. 2). They occurred exclusively in the lungs where they were numerous. They are elongate, between the alveoli, probably in endothelial cells. They are sinuous with a

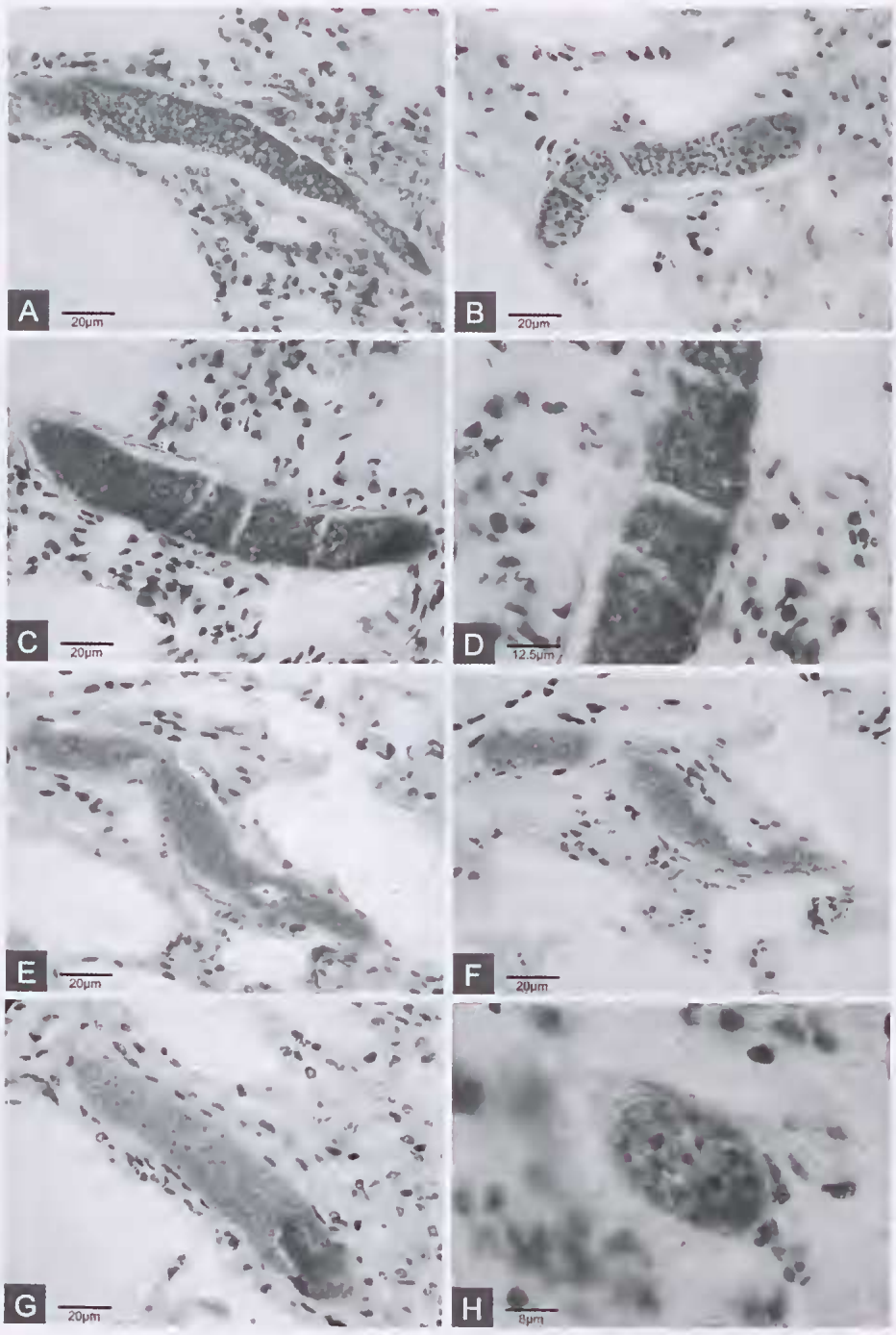


FIG 2. Photomicrographs of schizonts of *Johnsprentia copemani* sp. nov. in the lungs of *Pteropus alecto*. A-C, immature schizonts; D, detail of 'C' showing pseudosepta; E-F, immature schizont in consecutive sections showing the sinuous nature of the schizont; G, almost mature schizont with clear cytoplasm and punctiform nuclei; H, transverse section of young schizont showing large, dense, well defined nuclei.

pink border measuring 1.0-1.5 µm in thickness. Because of their sinuous form it is difficult to determine their precise size. The largest schizont in section (Fig. 2A) measured 160 µm long, 28 µm in maximum width and was seen in 14 sections 5 µm in thickness. Very young schizonts (Fig. 2H) have a dark blue cytoplasm, with large, dense chromatin masses which fragment progressively as the schizont matures. Individual nuclei are initially relatively large with dense chromatin and a well-defined outline (Fig. 2E, F). The centre of the nucleus becomes clear and pink, the chromatin forms aggregations of variable shape around the clear or pink centre. In some schizonts, pseudosepta are seen (Fig. 2B-D). A fully-developed schizont (Fig. 2G) measured 140 µm long and 26 µm wide, the cytoplasm was a clear blue, finely granular with small punctiform, uniformly distributed nuclei. There was no histomacrophagocytic reaction around the parasite. No schizonts were found in hundreds of sections of other organs of this bat (liver, spleen, kidney).

## DISCUSSION

In cases of polyparasitism, such as is observed in flying foxes in Queensland, it can be difficult to link the tissue stages with the corresponding gametocytes and it is necessary to connect histological observations on the tissues with those made on the erythrocytes. In the case of the species described here, we were fortunate to be able to study one bat at the commencement of an infection with only one type of gametocyte and schizont. Their morphology was uniform and therefore we are convinced that they belong to a single species.

The distinctive features of this parasite are primarily those of the gametocyte, in which the nucleus is elongated along the periphery of the cell and part of the cytoplasm is also concentrated at the periphery where it forms a dense, chromophilic band. These characteristics have not been reported in any other species described from *Pteropus* or any other bats, and have not been observed in other haemoproteids from mammals. In addition, the schizonts are exclusively pulmonary, elongated and devoid of colloid.

They are differentiated from the other genera of haemoproteids by the following characters: *Hepatocystis* (Laveran 1899) parasitic in numerous groups of mammals (primates, bats, Sciuridae, hippopotamus, tragulids): the classical merocysts of *Hepatocystis* develop in hepatocytes, are generally very large (can attain 2 mm in diameter in primates), frequently expand into neighbouring tissues and secrete a colloidal substance inside or around the schizont; the schizonts of *Johnsprentia* are localised in the lungs and are much smaller, are compact and elongate and do not secrete colloid.

*Nycteria* Garnham & Heisch (1953), parasites of micro-bats in which the schizonts localise in hepatocytes and are rounded or lobed, while those of *Johnsprentia* localise in the lungs and are elongate.

*Polychromophilus* Dionisi (1899), the schizonts are, (as with those of *Johnsprentia)* pulmonary, but by contrast, those of *Polychromophilus* occur equally frequently in other organs such as the kidney, spleen, liver and even the adrenals. The schizonts of *Polychromophilus* are ellipsoidal and surrounded by a thick, brightly pink capsule, their cytoplasm is poorly chromophilic and their nuclei are small, even in young forms. The schizonts of *Johnsprentia* are exclusively pulmonary, are elongated and botuliform, their host cell is poorly visible, their limiting membrane is thin, the cytoplasm of the immature stages stains intensely blue with Giemsa and their nuclei are relatively large.

*Bioccala* Landau *et al.* (1980), parasites of microbats. The small schizonts of *Bioccala* (Mer & Goldblum 1947; Landau *et al.* 1980) are disseminated throughout the body and do not resemble the current parasite in any way.

*Biguetiella* Landau *et al.* 1984, again has schizonts that are very small and intra-hepatocytic, bearing no resemblance to the current parasite.

The schizonts of *Rayella* (Dasgupta 1967), form rounded, intrahepatocytic groups, very different from the isolated, elongated pulmonary schizonts of *Johnsprentia*.

*Dionisia* Landau *et al.* 1980. The schizonts of *Dionisia* are rounded or oval, small in size and localise in the lumen of the hepatic vasculature, in a host cell which is hypertrophic and surrounded by a thick capsule. The schizonts of *Johnsprentia* do not possess any of these characters.

In fact, the most similar schizonts morphologically are those of *Parahaemoproteus* of birds, such as those described from the musculature of *Psittacula roseata* in Thailand, and which are transmitted by *Culicoides* (Miltgen *et al.* 1981). Although larger, as they can attain a size of 900 µm, they are similar to those of *Pteropus* in being elongated, botuliform, highly chromophilic, with pseudosepta, have a thin outer membrane and their host cell is scarcely visible. There is, in neither species, an accumulation of macrophages prior to the rupture of the schizont.

The obvious similarity between the schizonts of *Johnsprentia* and those of *Parahaemoproteus desseri* could indicate a close relationship, one parasitic in mammals, the other in birds. Such a phenomenon is possible since we know that, in the case of the haemosporidians, the ancestral host is an invertebrate, probably *Culicoides* in this case, and not the vertebrate host. However, this hypothesis is unlikely as the morphology of the gametocytes is of greater phylogenetic value than that of the schizonts, which, in this case, suggests that the two taxa are quite different phylogenetically.

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